36. (new) The method of claim 20, wherein the ribonuclease is ribonuclease T1 or binase.

37. (new) The method of claim 20, wherein the first and second polypeptides each comprise a separate subsequence of a single functional ribonuclease polypeptide.

REMARKS

With this amendment, claims 1-4, 6, 7, 11-18, 20, 21, and 25-37 are pending in the present application and are currently under examination. Applicants would like to thank Examiners Zaghmout and McElwain for the helpful interview of October 29, 1999 with Applicant's attorney Annette Parent at the Patent and Trademark Office, during which the enablement rejection described below was discussed. For convenience, the Examiner's rejections are addressed in the order in which they were presented in the June 7, 1999 Office Action.

The Invention

The present invention provides for the first time a two-component system to produce a lethal effect in plant cells. In this system, two polypeptides are expressed in a plant cell. The polypeptides are encoded by expression cassettes located at the same locus on each of two homologous chromosomes. One expression cassette comprises a first promoter operably linked to a first polynucleotide sequence, with a recombinase site between the first promoter and the first polynucleotide sequence. The second expression cassette comprises the first plant promoter inoperably linked to the first polynucleotide due to the presence of a second expression cassette, flanked by two recombinase sites, between the first promoter and the first polynucleotide. The second expression cassette comprises a second promoter operably linked to a second polynucleotide.

Each expression cassette of the invention is individually functional, but the product of each cassette alone does not provide the desired lethal effect. The combination of

the two polypeptides from the individual expression cassettes is required for producing the lethal effect. The first and second polypeptides can either be separate functional polypeptides from distinct loci, or nonfunctional polypeptide subsequences that together produce a single functional polypeptide.

Status of the Specification

The specification contained a typographical error that is corrected by this amendment. No new matter is introduced by this amendment.

Status of the Claims

Claims 1 and 14 have been amended to recite "wherein at least the first or the second plant promoter is a non-constitutive promoter." This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 6, lines 15-17.

Claims 1 and 14 have been amended to recite "wherein at least the first or the second polynucleotide encodes an amino acid sequence from a nuclease." This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 16, line 12.

Claims 6 and 20 have been amended to recite "wherein at least the first or the second polynucleotide encodes an amino acid sequence from a ribonuclease." This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 2, line 30.

Claims 13 and 27 have been amended to recite "nuclease polypeptide." This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 16, line 12.

New claims 28 and 33 have been added, which recite that both the first and the second promoters are non-constitutive promoters. These claims add no new matter. Support for these claims can be found, e.g., in the specification on page 6, lines 15-17.

New claims 29 and 34 have been added, which recite promoters having "overlapping specificities." These claims add no new matter. Support for these claims can be found, e.g., in the specification on page 19, lines 17-18 and page 11, line 1.

New claims 30 and 35 have been added, which recite a first or a second promoter that is "seed coat-specific." These claims add no new matter. Support for these claims can be found, e.g., in the specification on page 11, line 12.

New claims 31 and 36 have been added, which recite "ribonuclease T1 or binase." These claims add no new matter. Support for these claims can be found, e.g., in the specification on page 16, lines 9-11.

New claims 32 and 37 have been added to recite that the "first and second polynucleotides each comprise a separate subsequence of a single functional ribonuclease polypeptide." These claims add no new matter. Support for these claims can be found, e.g., in the claims 13 and 27 as filed and in the specification on page 16, lines 19-23.

Rejection under 35 U.S.C. § 112, first paragraph: written description/enablement

A. Written Description

Claims 1 and 14 were rejected as allegedly containing subject matter that was not described in the specification. The rejection states that Applicants have not disclosed a plant "where the inserted genes can be transcribed by any promoter." Office Action, page 3. The rejection further notes that if both nucleic acids in the expression cassette are driven by constitutive promoters, all cells would be killed and no transgenic plants would be produced. *Id.* It appears that the rejection relates to a concern regarding inoperable embodiments, i.e., that if certain combinations of promoters are used, no transgenic plants will be produced.

Applicants respectfully traverse the rejection. The standard for determining compliance with the written description requirement is whether "the description clearly allows persons of ordinary skill in the art to recognize that he or she invented what is claimed." MPEP § 2163.02; *In re Gosteli*, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). The specification describes that the claimed invention is used to kill selected cells, and describes a variety of promoters to be used to achieve that result. Furthermore, one of skill in the art would know to avoid certain combinations of promoters as inoperable embodiments. Finally, the claims have been amended to recite that either the first or the second promoter is a "non-constitutive promoter."

1. The specification describes combinations of promoters for use in the invention

The specification describes that the claimed invention is used to kill selected cells of a plant (*see, e.g.*, specification, page 3, lines 16-17). The present specification also fully describes the use of a number of different promoters, including constitutive and non-constitutive promoters such as inducible and tissue-specific promoters, to achieve this result (*see, e.g.*, specification, page 10, line 30 to page 11, line 20). For example, the specification describes that one may use a combination of promoters whose expression overlaps, e.g., a constitutive promoter and a tissue-specific promoter, or a combination of two tissue-specific promoters, or a combination of inducible promoters. As Applicants have explicitly described the use of a variety of promoters in the specification, the written description rejection is inappropriate.

2. One of skill in the art would be able to avoid inoperable embodiments

It appears that the rejection is concerned about possible inoperable embodiments, in which a plant would not be produced due to complete lethality. For example, the Office Action describes a situation where two constitutive promoters are used and expressed in every single cell of the plant. Office Action, page 3. In another example, the rejection is concerned that if tissue-specific promoters are used "there is a likelihood that promoters in both expression cassettes will be expressed in the same tissue at the same time where transgenic plants will not be produced." *Id.*

Applicants first note that the claims have been amended to recite that "at least the first or the second promoter is a non-constitutive promoter." The claims therefore exclude the first situation described by the rejection, where the use of two constitutive promoters leads to complete lethality.

In addition, one of skill in the art would be able to select suitable promoters and avoid inoperable embodiments of the type described by the rejection. As described by the Court of Customs and Appeals:

> [M]any patented claims read on vast numbers of inoperative embodiments in the trivial sense that they can and do omit 'factors which must be presumed to be within the level of ordinary skill in the art.' . . . There is nothing wrong with

> this so long as it would be obvious to one of skill in the art how to include these factors in such manner as to make the embodiment operative rather than inoperative. *In re Cook and Merigold*, 169 USPQ 299, 302 (C.C.P.A. 1971) (quoting *In re Skrivan*, 166 USPQ 85, 88 (C.C.P.A. 1970)).

Thus, one of skill in the art would know to select promoters that produce lethal effects in certain cells of a plant. Even if one assumes, *arguendo*, that complete lethality might occur when using two particular promoters, this situation does not indicate that the claimed invention is not described by the specification. One of skill in the art would clearly understand that another transgenic plant could be created in its place, using different promoters as described by the specification. Applicants therefore respectfully request that the rejection be withdrawn.

B. Enablement

Claims 1-27 were also rejected for allegedly lacking enablement, for the reasons described above, i.e., that a plant would not be produced if the promoters were both expressed in all cells. Applicants again respectfully note that the claims have been amended to recite that at least one of the promoters is a non-constitutive promoter. To the extent that the rejection applies to the amended claims, Applicants traverse for the reasons described above. One of skill in the art would be able to identify inoperative combinations of promoters with only routine experimentation, and would be able to substitute operative combinations of promoters in their place. Applicants therefore respectfully request that the rejection be withdrawn.

Rejection under 35 U.S.C. § 112, first paragraph: enablement

Claims 1-27 were rejected as allegedly not enabled by the specification. The rejection states that the "many functions in plants are controlled by more than two genes." Office Action, page 5. The rejection further states that "it is unpredictable whether the genes . . . will be expressed as expected once they are integrated into the genome." *Id.* Effectively, the rejection appears to argue that one of skill in the art would not be able to create transgenic plants, using any gene that is lethal to the cell, without undue experimentation.

Applicants have amended the claims to recite that at least one of the expression cassettes encodes amino acid sequences from a nuclease, whose expression produces the

desired lethal effect in particular cells. However, to the extent that the rejection remains applicable to the claims as amended, Applicants respectfully traverse. Methods of transforming plant cells are well known in the art, and the specification provides adequate guidance for transforming plants with the nuclease constructs of the invention. One of skill in the art would therefore be able to practice the claimed invention with, at most, only routine experimentation.

A. The amended claims recite polynucleotides encoding a sequence from a nuclease

Applicants respectfully note that the claims have been amended to recite that at least the first or the second polynucleotide encodes an amino acid sequence from a nuclease. The claims, therefore, are now directed to transgenic plants comprising polypeptides that form a nuclease, e.g., a ribonuclease or a deoxyribonuclease, which is lethal to the plant cell. Applicants submit that it would be routine for one of skill in the art to make a transgenic plant expressing nuclease polypeptides or polypeptide fragments. As described in the specification, many nucleic acid sequences are known for members of the colicin (deoxyribonuclease) and ribonuclease families, and new sequences from these conserved families could be easily identified using routine methodology (*see, e.g.*, specification at page 15). In addition, nucleases have a known mode of action--degradation of RNA or DNA--and so the Examiner's concern regarding modulation of plant cellular functions controlled "by more than two genes, such as genes which control photosynthesis, germination, and respiration" is not relevant to the claims as amended. It would therefore be routine to transform a plant with the nuclease constructs of the invention.

B. Plant transformation is well known in the art and described in the specification

In the field of plant biotechnology, transformation of a variety plant species with a heterologous gene of choice is merely routine. In addition, the specification provides guidance for transforming plant species. The assays of the specification, together with standard methodology known to those of skill in the art, therefore provide adequate guidance for using the claimed methods to express a heterologous nuclease in a wide variety of plants.

The proper test of enablement is "whether one skilled in the art could make or use the claimed invention from the disclosure in the patent coupled with information known in the art without undue experimentation." *See, e.g.*, MPEP § 2164.01. As identified by the Patent Office and the Federal Circuit, whether undue experimentation is required by one skilled in the art to practice the invention is determined by considering factors such as the breadth of the claims, the level of one of ordinary skill, amount of guidance presented in the application, and the presence of working examples. *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int. 1985); *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). As described in *Wands*, "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should precede." *Wands*, 8 USPQ2d at 1404 (quoting *In re Jackson*, 217 USPQ 804 (Bd. Pat. App. & Int. 1982).

At the time of the present invention, transformation of plant species with a variety of expression vectors encoding a particular heterologous gene, such as a nuclease, was well within the means of one of skill in the art, without undue experimentation. For example, suitable techniques for plant transformation and regeneration are described, e.g., Klee, *Ann. Rev. of Plant Phys*, 38:467-486 (1987); Weising *et al., Ann. Rev. Genet.* 22:421-477 (1988); *Handbook of Plant Cell Culture* (1983); and Binding, *Regeneration of Plants* (1985). Such techniques are standard methodology and are well known to those of skill in the art.

The present application also provides considerable direction and guidance for practicing the claimed invention, as the specification describes methods of transforming plant cells and regenerating plants. For example, the specification describes how to perform both transient and stable transformation methods. Numerous different methods for plant transformation are described, including methods such as electroporation, microinjection, ballistic methods, *Agrobacterium* transformation, and PEG precipitation (*see, e.g.*, specification, pages 8-9). In addition, the specification also describes regeneration techniques for generating plants from transformed plant cells (*see, e.g.*, specification, page 19). Applicants thus submit that transformation of a plant with expression cassettes encoding a nuclease requires, at most, only routine experimentation.

C. Use of a nuclease to produce the lethal effect is predictable

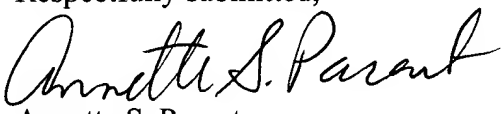
The rejection also appears to argues that the use of a variety of genes to produce a lethal effect is not predictable. According to the rejection, "[I]t is essential to determine that the claimed method is enabled as a method of modulating cellular functions in a plant." Office Action, page 4. Again, Applicants respectfully point out that the claims have been amended to recite expression of a nuclease. Nucleases have a known mechanism of action, so their effect in the plant cell is predictable--RNA or DNA essential for cell survival is degraded. The Examiner's apparent concern regarding control of complex functions such as "photosynthesis, germination and respiration" is therefore moot.

Furthermore, Applicants submit that the present specification teaches how to make and distinguish operative nuclease embodiments, with only routine experimentation. For instance, the specification teaches how to make and use the two-component system of the invention with separate monomers that form, e.g., a dimeric ribonuclease (*see, e.g.*, specification, page 13). The specification teaches how to make and use nucleases activated by a transactivator protein (*see, e.g.*, specification, page 11, line 27 to page 12, line 18). The specification also teaches how to make and use two non-functional nuclease subsequences that together make a single functional nuclease, e.g., using partial proteolysis, sequence conservation-based design, or structure-based design (*see, e.g.*, specification, pages 15-17). The specification also provides an example where the ribonuclease barnase has been cleaved into two subsequences, which together form a functional enzyme (*see, e.g.*, specification, pages 16-17). Finally, assays for nuclease cytotoxicity are discussed, e.g., in the specification on page 17. Thus, the specification provides sufficient support to enable one of skill in the art to use a nuclease in the methods of the claimed invention. Accordingly, Applicants respectfully request that the rejection of claims 1-27 be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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